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CHANGES IN THE NUTRITIONAL, ANTINUTRITIONAL AND ANTIOXIDANT CHARACTERISTICS DURING DEVELOPMENT AND RIPENING OF JAMAICA CHERRY *(MUNTINGIA CALABURA* L.*)*

T.GAYATHRI, V.RENJIMA, K.MURUGAN and T.C. KIŞHOR MOHAN
Plant Molecular and Biochemistry Lab., Department of Botany, University College, Thiruvananthapuram- 695 034, Kerala, India.
**Email:* drtckishor@yahoo.co.in*

Many wild plants make significant contribution to diets of tribal people allover the world. Unfortunately a few studies have been carried out to evaluate the potential nutritional and health benefits of such diets. In the present study the small delicious fruits of Jamaica cherry were analysed for their proximate composition including mineral constituents, antinutritional and antioxidant characteristics at various stages of fruit maturation. The proximate analysis of ripe fruits revealed moisture (78%), total ash content (5.67%), crude fibre (0.972%), total sugar (87mg/g), crude protein (10mg/g), and fat content (17.5 mg/g FW). Mature fruits were found to be rich source of essential minerals. In addition to the major nutrients fruits contained some antinutritional characteristics such as tannins, ranged (390μg to 97.2μg/100g tissue), phytic acid (6.25μg and 2.5μg/100g) and total phenol content (8.88 mg to 3.93mg /g FW) at 2WAP and 8WAP respectively. The value of antinutritional factors was below the standard value of World Health Organization (WHO). Appreciable quantities of β-carotene (113.35μg/g), ascorbic acid (588.23μg/g) and tocopherol (82.67μg/g tissue) were observed in mature fruits. Considerable variation was noticed in the level of β-carotene (18.69μg/g to 113.35μg/g); ascorbic acid (262.02mg/ 100g to 588.23mg/ 100g) and tocopherol content (58.77μg/g to 82.67μg/g tissue) at different phases of fruit maturation. The antioxidant potential strongly correlates with the total phenol content during the stages of fruit development. Subsequently the fruits were further analysed for fractionating the polyphenolics by RP-HPLC. A positive correlation was noticed between phenolic acids and total phenol content suggesting their role as precursors of many of the secondary metabolites of the plant. The presence of the phenolic acids such as caffeic, coumaric, chlorogenic, ferulic, gallic and vanillic acid increases further the antioxidant potential. The biochemical and analytical data clearly suggest that the fruits have enormous potential to meet the nutritional and antioxidant need in the diets of populations inhabiting in the remote parts of the world especially during the famine periods.

Keywords: Antinutritional factors; Antioxidants; Ascorbate; β-Carotene; Minerals; Phenolic acids; Phenols; Tocopherol; Wild food plants.

Introduction

The Jamaica Cherry, *Muntingia calabura* L., (Elaeocarpaceae) is an evergreen plant, indigenous to southern Mexico, Central America and tropical South America. It is widely cultivated in warm areas of the New World and in India, southeast Asia, Malaya, Indonesia, and the Philippines. This fast growing tree is rapidly spreading in different parts of India from north to south. In Kerala it is widely grown in home gardens and also along the highway sides as a shade tree. The fruits are small round, with red or sometimes yellow, smooth, thin, tender skin and light-brown, soft, juicy pulp, with very sweet, musky, somewhat fig-like flavor, filled with minute, yellowish seeds. The tiny delicious fruits popularly known as "poor man's fruit" are widely consumed by the Jamaican tribes.

Edible plants contribute significantly to the nutrition of inhabitants of rural areas of the world. With ever increasing pressure and fast depletion of natural resources, it has become extremely important to diversify the present day agriculture inorder to meet various human needs. The observed interest in search for alternative / additional food and feed ingredients is of paramount importance[1]. The nutritional value of food is primarily determined by their protein content, followed by carbohydrates and fats. With increasing interest in finding new alternative food source, the wild or unutilized plants receives more attention which offer a good scope to meet the increasing demands for vegetable protein, carbohydrates, omega-6 and omega-3 poly unsaturated fatty acids (PUFAs) and natural antioxidants such as provitamin A (β-carotene), vitamin C (ascorbic acid), vitamin E (α- tocopherol), polyphenols, flavonoides etc. Antioxidants are electron donors and act as free radical scavengers by reducing oxidative damage and the accompanying damaging effects in the body. Today we know that these compounds are essential for normal growth and development and may play an important role in the prevention of coronary artery disease, neurodegenerative disorders and cancer caused by free radicals and reactive oxygen species[2]. The major problem encountered with the exploitation of the potential of many wild plants was the occurrence of some antinutritional factors like tannins, protease inhibitors, saponins, lectins, phytic acid, goitrogens, allergens, antivitamins and several phenolic derivatives in higher or lower levels along with nutritional components. Antinutritional factors refer to naturally occurring compounds that act to reduce nutrient utilization and food intake. All of these are allelochemical substances of varied nature that react with food substances in different ways. For example, tannins and saponins have metabolic effects on protein digestibility and intestinal permeability[3]. Many cultivating and wild plants belonging to different families are known to possess one or more of these substances. Seeds of many legumes even though they are rich in protein, contain a large number of antinutritional substances, which hinder free nutritional utilization in monogastric animals and humans. Little is known about the nutritional, antinutritional and antioxidant status of Jamaica cherry. The studies so far conducted on *Muntingia calabura* have been largely confined to the antibacterial activity of leaf extract and the medicinal property of stem bark. So the present investigation is an attempt to understand the fruit as a whole with special reference to the nutritive, antinutritive and antioxidant characteristics.

Materials and Methods

Plant material: Fruits at different stages of maturation were collected from the college campus. The age of the fruit was calculated in weeks from the date of pollination and is expressed in weeks after pollination (WAP).

Chemicals: All biochemicals used were obtained from Sigma Chemical Co., St. Louis, MO, USA and other chemicals were of analytical grade.

Proximate composition analysis: The fruits were analyzed for proximate composition by using standard methodologies. Moisture and ash were determined according to AOAC[4], crude fibre content was determined by the method described by Pearson[5]. Carbohydrates and reducing sugars were determined by the method of Mohan and Janardhanan[6]. Crude protein was obtained by multiplying the total nitrogen content by a factor value proposed by Pearson[5].

Amino acid analysis: The fruit samples were hydrolyzed in 6 N HCl for 18 hours at 110°C. The hydrolysate was filtered and analyzed by HPLC[7]. Since tryptophan is destroyed during hydrolysis, the method involving the hydrolysis of samples in 5 N NaOH was used for tryptophan.

Mineral analysis by atomic spectroscopy: The major and minor nutrients were estimated from the fruit tissues by Atomic Spectroscopy[8].

Estimation of total phenols: Total phenol content of fruit tissues was estimated by the method of Mayr *et al*[9]. The total phenols/g tissue was calculated from the standard graph.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) of phenols: Phenolic components of extracts were separated using HPLC following the method of Beta *et al*[10]. Standard phenolic acids such as gallic, vanillic, p-hydroxybenzoic, ferulic, chlorogenic sinapic, para coumarate and cinnamic acids were injected into the column separately. Comparing with the retention time of the standard phenolic acids in the sample was identified. Height of the peaks was taken for quantification.

Estimation of ascorbate: Ascorbate is extracted and quantified as per the methodology of Ranganna[11].

Estimation of β-carotene and tocopherol: The carotene and tocopherol content of fruit samples was estimated by extracting the tissues by soxhlet method using the solvent Hexane[12] and the absorbance were recorded at 429 nm and 292 nm respectively.

Antinutritional factor analysis: A quantitative analysis of tannins was carried out spectrophotometrically using Folin- Dennis reagent[13]. Extraction was done with methanol/water. Tannic acid was used to prepare the standard graph. Total phenol contents of fruit tissues were estimated by the method described earlier. The total phenols /g tissue was calculated from the standard graph. Phytic acid content was determined by the method of Ravindran and Ravindran[14].

Estimation of In vitro antioxidant activity: Antioxidant activity was estimated as per assay method of Benzie and Strain[15]. 100 μl of ethanolic extract was added to 3 ml of FRAP reagent (10 mM 2,4,6- tripyridyl S – triazine (TPTZ) in 40 mM HCl and 20 mM ferric chloride in 300 mM sodium acetate buffer, pH 3.6 in the ratio of 1:1: 10 (v/v) and mixed thoroughly and absorbance noted after 4 min, at 593 nm against water blank. Calibration was against a standard curve (50- 100μM) ferrous ion produced by the addition of freshly prepared ammonium ferrous sulphate. Values were obtained from three replications and expressed as μmol FRAP g^{-1} fresh weight.

Results and Discussion

The proximate analysis of mature fruits reveals moisture

(78%), total ash content (5.67%) and crude fibre (0.972%). The results of the proximal composition of fruits at various stages of development i.e. 2WAP to 8 WAP, are presented in Figure 1 which indicates appreciable variation in crude protein content, total sugar, crude fat and moisture content. The crude protein values ranged between 1.82 mg/g and 10 mg /g fresh weight (FW). Fully ripened fruits showed the highest value (10 mg/ g) with regard to protein content, which is greater than the value, estimated in grapes (8 mg / g)[16]. The total sugar varied from 28 mg / g FW in young fruits at 2WAP to 87 mg / g in mature fruits at 8WAP. The total sugar content can be comparable to some common edible fruits such as mango (8.51g /100g), orange (8.2g / 100g) and grapes (13.2g /100g)[16]. Remarkably higher level of sugar in fresh ripened fruits underscores their importance as a potential source of this vital nutrient. The crude fat content of the fruits varied between 12.5mg /g at 2WAP and 17.5 mg/ g FW at 8WAP. A progressive increase in fat content was observed at various stages of fruit maturation.

Mineral analysis: Results of the mineral constituents in ripe fruits are presented in Table 1. The fruits contain appreciable levels of nutritionally valuable minerals. Potassium was the most abundant element (2.15%) among the mineral components. The ratio of Na/K is 0.71%. The ratio of Na / K in the human body is of great significance as it helps in the regulation of high blood pressure[17]. The values of other macronutrients sodium (1.52%), phosphorous (0.396%), calcium (1%), magnesium (0.39%) and sulphur (0.283%) were also significant. The Ca/P ratio is 2.53%. High Ca/P ratio helps to increase the absorption of Ca in the small intestine[17]. It is also evident from the data that the fruits are rich source of essential micronutrients such as iron (70ppm), manganese (26ppm), zinc (45ppm) and copper (14ppm). Cu, Zn and Mn are essential components of several enzymes that catalyze cellular oxidative- reduction reactions and play a vital role in collagen synthesis and iron mobilization. Moreover these trace elements protect the body from cellular damages by scavenging reactive oxygen species directly or indirectly through antioxidant enzymes.

Table 1. Mineral constitution in ripe fruits .

Elements	%/ ppm
Na	1.52
P	0.396
K	2.15
Ca	1.00
Mg	0.39
S	0.283
Fe	70ppm
Mn	26ppm
Zn	45ppm
Cu	14ppm

Amino acid composition: The data on amino acid profile is given in Table 2. It is observed that glutamate and aspartate are the abundant amino acids and make up to 11.4 mg / g tissue on an average basis with a percentage of 38.23. Lysine and phenylalanine are essential amino acids necessary for the synthesis of new protein for growth and repair. The total essential and non-amino acid in the sample is 6.41 mg / g (21.5 %) and 23.41 mg / g (78.5%) respectively. The total neutral (55.9%), acidic (38.23%) and basic (5.84%) amino acids values suggesting that the proteins are probably acidic in nature. Therefore the fruits can be considered as good diet that can provide the required essential amino acids.

Table 2. Amino acid composition in mature fruit of *Muntingia*

Amino acids	mg/g tissue
Lysine	0.2
Arginine	1.3
Histidine	0.24
Glutamate	4.9
Aspartate	6.5
Tyrosine	0.29
Leucine	0.55
Tryptophan	1.09
Serine	1.1
Glycine	4.9
Alanine	2.1
Cysteine	3.4
Methionine	0.1
Valine	0.09
Isoleucine	1.9
Phenylalanine	0.6
Threonine	0.34
Proline	0.22

Antinutrients : Fig. 2 a, b and c represent the results of few antinutritive constituents such as total free phenolics, tannins and phytic acid in fruits at various stages of maturation. The presence of antinutritional factors adversely affects the nutritional qualities of many wild and cultivated fruits. A preliminary evaluation of some of these factors was made in *Muntingia.* A significant decrease in phytic acid content (6.25 μg /100g to 2.5μg / 100g) was noticed at different stages of fruit ripening. The quantity of tannin in fresh fruits ranged between 390μg (2WAP) and 97.2μg / 100g (8WAP) and the total phenolic content ranged from 3.93 mg / g to 8.88 mg / g. Phenolic compounds decrease the digestibility of proteins, carbohydrates and the availability of vitamins and minerals. They lower the activity of digestive enzymes such as amylase, trypsin, chymotrypsin and lipase and may cause damage to the digestive tract[18]. Interestingly the low level of antinutrient factors in the ripened fruit increases the

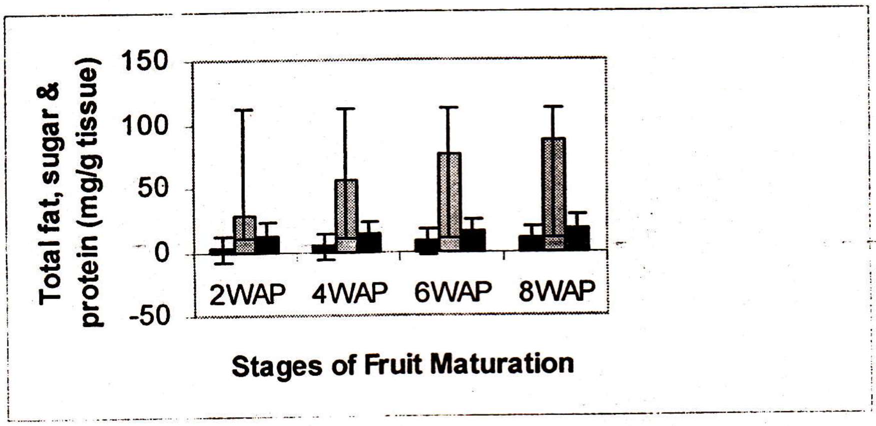

Fig. 1. Displays total fat, sugar and protein in different stages of fruit maturation.

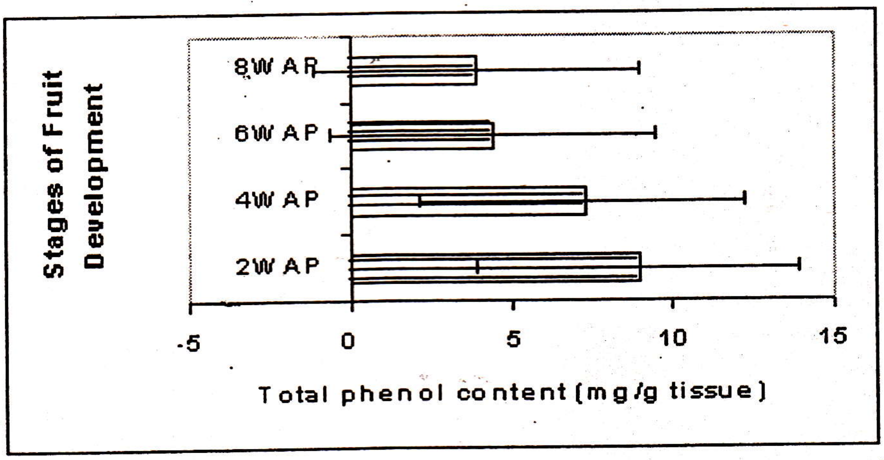

Fig.2a. Bar diagram shows the total phenol content in different stages of fruit maturation

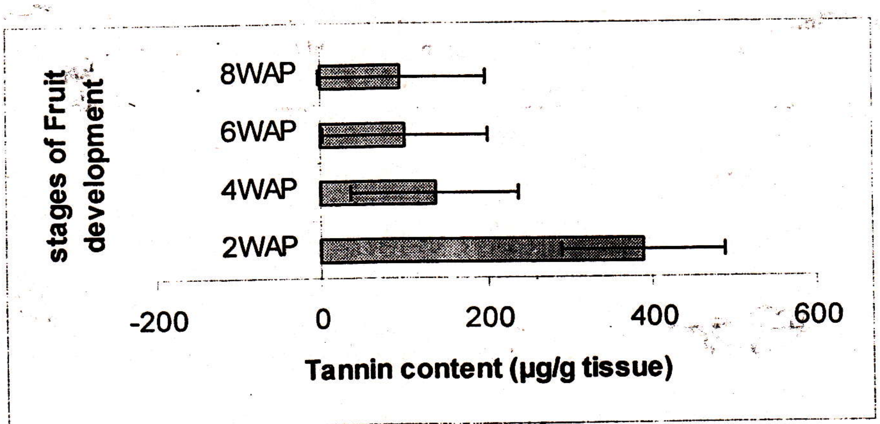

Fig. 2b. Bar diagram shows the total tannin content in different stages of fruit maturation.

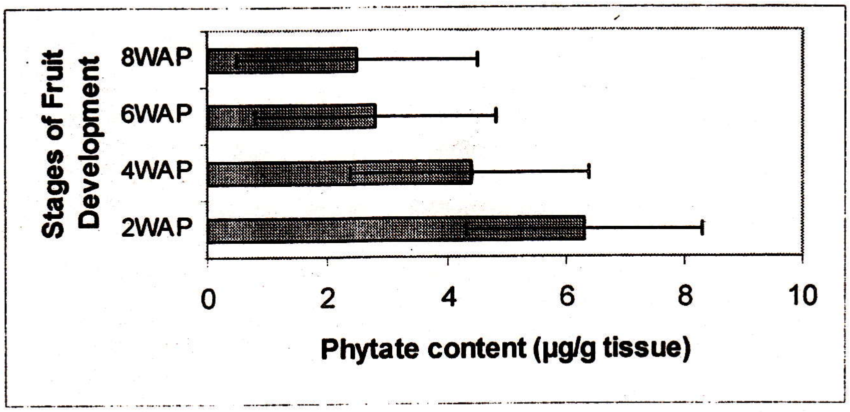

Fig. 2c. Total phytate in different stages of fruit development.

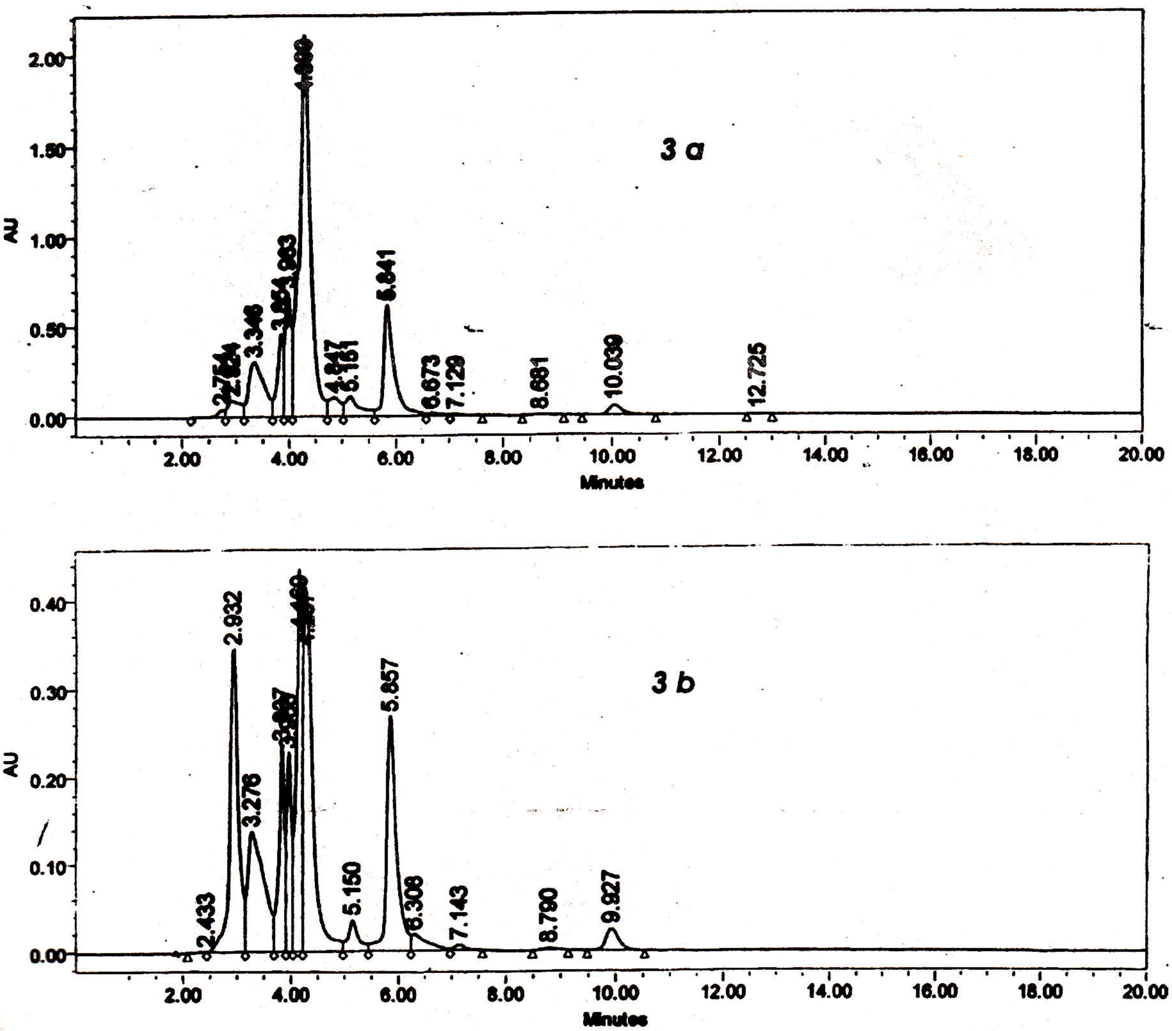

Fig. 3a & 3b. HPLC Chromatogram of *Muntingia* fruits at different stages of maturity. 3a. 2 WAP, 3b. 8 WAP.

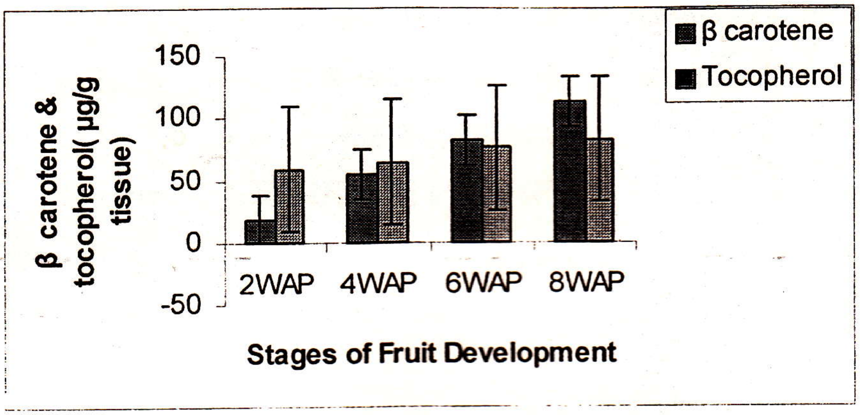

Fig. 4a. β–carotene and tocopherol content in different stages of fruit development.

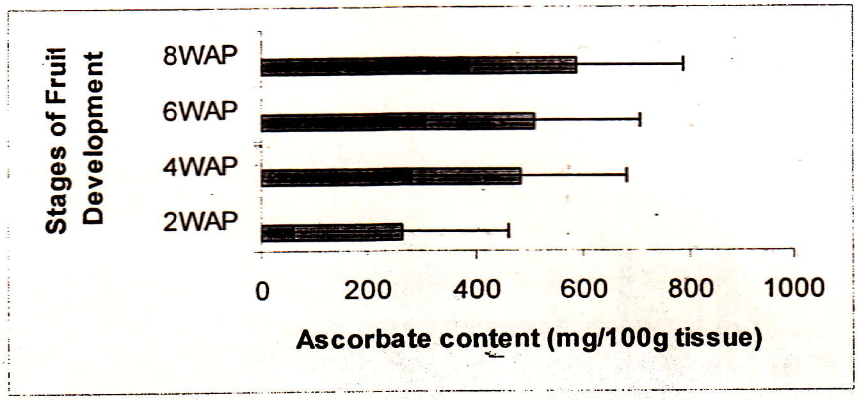

Fig. 4b. Ascorbate content in different stages of fruit development.

nutritional potential of this fruit. Subsequent to the estimation of total phenol it was fractionated to know the profile of phenolic acids in the young and mature stage of the fruit (Fig. 3a & b) (Table 3). The major phenolic acids in the mature fruits are caffeate, coumarate, chlorogenate and gallate. These phenolic acids are effective antioxidants because they scavenge reactive oxygen species, trap nitrate and prevent formation of mutagenic N- nitroso compounds and also have metal chelating properties[19].

Table 3. Phenolic acids in different stages of fruit development.

Phenolic acids	2WAP(µg/g tissue)	8WAP(µg/g tissue)
Caffeate	327.2	73.8
Coumarate	39.92	18.96
Chlorogenate	884.3	415.1
Ferrulate	104.9	ND
Gallate	112.4	290.6
Paracatechol	33.97	ND
Vanillate	91.6	ND

Antioxidants: Fig. 4 a and b displays the antioxidant characteristics such as pro-vitamin A (β-carotene), vitamin C (ascorbic acid) and vitamin E (tocopherol) in fruits at various stages of development. Carotenoids are pigments found in most fruits and vegetables. The human body does not produce carotenoids; therefore they need to be obtained through diet. β-Carotene is the most important and frequently studied among all carotenoids. In the present investigation β-carotene content ranged from 18.69 µg / g at 2WAP to 113.35 µg / g FW at 8WAP (Fig. 4a), higher than the value reported in mango (56 µg / g). Carotenes have a positive effect on the immunological system and protect the skin from ultraviolet radiation[20]. In addition to the pro -vitamin A activity, β-carotene has been found to reduce risks of certain cancers especially lung cancer[20]. The level of tocopherol content ranged from 58.77µg / g to 82.67 µg / g and ascorbic acid from 262.02mg/ 100g to 588.23 mg / 100g at 2WAP and 8WAP respectively. Phenolic compounds are perhaps the largest group of phytochemicals that have shown disease preventing and health promoting effects due to their antioxidant activity. Tocopherol is also a phenolic compound that plays a significant role as an antioxidant to protect polyunsaturated fatty acids (PUFAs) and other components of cell membrane and low-density lipoprotein (LDL) from oxidation, therefore preventing heart diseases[20]. Ascorbic acid is perhaps the most important antioxidant in extra cellular fluids. It was found to be the most effective in inhibiting lipid peroxidation initiated by peroxyl radical initiator among several types of antioxidants including α-tocopherol. In addition, ascorbic acid is an effective radical scavenger of superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. Many studies have shown that an adequate intake of vitamin C is effective in lowering the risk of developing cancers and cardio vascular diseases[21].

In vitro antioxidant activity (AOX) was measured by FRAP assay exhibited sound variation between the initial and final stages of fruit maturation. The AOX activity after 2WAP is 1618μmol while 8WAP the value reduced to 761μmol. In order to further assess the relationship between TPC and AOX activity Pearson's coefficient of correlation was worked out. Interestingly the TPC shows a positive and strong relationship with the antioxidant activity ($R^2 = 0.811$) suggesting that phenolic compounds are key determinants of antioxidant activity in foods. Regression analysis revealed that for every increase in phenolic content there was a corresponding 0.28 increase in AOX activity. Higher correlation between phenolics and antioxidant activity, confirms the earlier results of several vegetables[22].

The present study indicates that Jamaican cherry can be classified as source of protein with valuable minerals and essential aminoacids. It also reveals the suitability of diets for hypersensitive people since Na/K ratio is less than one. Similarly the Ca/P ratio is also ideal. The emergence of such underutilized fruits can be ascribed to multiple factors such as growing populations, increasing health consciousness and consumers in general and elevating health care cost. Further research work is warranted on *in vitro* and *in vivo* experiments related to biological evaluation and health promoting aspects one needed.

References

1. Nieman D C, Butterworth D E and Nieman C N 1992, Nutrition. Wm. C. Brown Publishers Duduque.
2. Ponka R, Fokou E, Leke R, Fotso M, Souopgui J, Achu Mercy B and Tchouanguep Mbiapo F 2005, Methods of Preparation and Nutritional Evaluation of Dishes consumed in a Malaria Endemic Zone in Camerooon (Ngali II). *Afr. J. Biotechnol.* 4 (3) 273-278.
3. Siddhuraju P and Becker K 2001, Effect of various domestic processing methods in antinutrients and *in vivo* protein and starch digestability of two indigenous varieties of Indian tribal pulse, *Mucuna puriens var. utilis. J. Agri. Food. Chem.* 49 3058 – 3067.
4. AOAC 1980, Official Methods of Analysis. 13th ed. William Horwitz (ed.) Washington, DC: *Association of Official Analytical Chemists* 7 56-132.
5. Pearson D 1976, Chemical analysis of foods. 6th edn. Churchill Livingstone, London.
6. Mohan V R and Janardhanan K 1995, Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna. Food Chem.* 52 275-280
7. Singhai B and Shrivastava S K 2006, Nutritive value of new chick pea (*Cicer arietinum*) varieties. 4 (1) 48 – 53.
8. Bharghava and Raghupathy 1993, Analysis of plant material for macro and micro nutrients. *In* : Methods of analysis of soils, plants, water and fertilizer. Tandon, HLS (ed.), FDCO, New Delhi.
9. Mayr V, Treutter D, Santos-Buelga C, Bauer H, Feucht W 1995, Developmental changes in the phenol concentration of golden delicious apple fruits and leaves. *J. Agric. Food Chem.* 38 1151-1155.
10. Beta T, Rooney LW, Marovatsanga LT, Taylor JRN 1999, Phenolic compounds and kernel characteristics of Zimbabwean sorghums. *J. Sci. Food Agri.* 79 1003-1010.
11. Ranganna S 1997, *In* : Manual analysis of fruit and vegetable product, 9th edition, Tata Mc Graw Hill, New Delhi.
12. IUPAC 1987, Standard methods for the analysis of oils, fats and derivatives. Blackwell Scientific Publications, Oxford.
13. Singleton V L and Roos JA 1965, Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *Am. J. Enol. Vitric* 16 144-149.
14. Ravindra V and Ravindran G 1988, Nutritional and antinutritional characteristics of *Mucuna (Mucuna utilis)* bean seeds. *J. Food Sci. Agri.* 46 71-79.
15. Benzine IF, Strain J 1999, Ferric reducing antoxidant power assay direct measure. *In: Methods Enzymology* 299.
16. Linder MC, Copper 1996, *In* : Ziegler EE, Filer LJ, *Present Knowledge in Nutrition.* 7th (ed.) Washington, DC : *Intr. Life Sci. Inst.* 307.
17. Conningham JJ 1998, Micronutrients as nutriceutical interventions in diabetes mellitus. *J. Am. Coll. Nut.* 17(1) 7-12.
18. Vadivel V and Janardhanan K 2000, Nutritional and antinutritional a compositin of velvet bean : an

underutilized food legume in south India *Int. J. Food Sci. Nutri.* **51** 279-287.

19. Vinson JA, Xuehui S, Zubik L, Bose P 2001, Phenolic antioxidant quantity, quality in foods and fruits. *J. Agri. Food Chem.* **49** 5315 -5319.
20. Heinonen MI, Ollilainen, Linkola EK, Varo PT, Koivistoinen PE 1989, Carotenoids in finish foods, vegetables, fruits and berries. *.J Agri. Food Chem.* **37** 655 - 659.
21. Taso R and Akhtar MH 2005, Nutraceuticals and functional foods: I. Current trend in phytochemical antioxidant research.*J. Food Agri. Environ.* **3** (1) 10-17.
22. Bravo I 1998, Polyphenol chemistry, directory sources, metabolism and nutritional significance. *Nutri. Rev.* **56** 317-325.